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BIOLOGICAL BULLETIN

TWO TRANSITIONAL STAGES IN THE DEVELOPMENT OF CYCLOPS SIGNATUS, VAR. CORONATUS.

A Preliminary Note.

ESTHER F. BYRNES.

While studying some of the inhabitants of a pool of spring water at Cold Spring Harbor, Long Island, during the months of July and August, I was perplexed by the constant occurrence of two comparatively large *Cyclops* that I was unable to identify as belonging to any known species.

The prevalent adult forms in the pool were *C. signatus* var. *tenuicornis* (*annulicornis*) and var. *coronatus* and *C. serrulatus*. That the forms in question bore no relation to *C. serrulatus* was quite evident. In certain characteristics they agreed with *C. signatus* but for some time I hesitated to associate them with *C. signatus* because of constant correlations in structure, and because the larger of the two forms was occasionally found to be sexually mature.

The smaller of the two cyclops, Plate VII., combined the following characteristics: the antennæ usually contained ten segments, but occasionally nine segments were present; the rami of the swimming feet were two-jointed, with an armature that indicated immaturity as follows:

First Foot.		Second Foot.	
Outer Ramus.	Inner Ramus	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
3 inner setæ,	5 inner setæ,	4 inner setæ,	5 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	Inner Ramus.	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
4 inner setæ,	4 inner setæ,	4 inner setæ,	3 inner setæ.

The rudimentary fifth foot was two-jointed, having an almost square basal segment bearing an immature seta on its outer distal angle, and a slightly elongated distal segment bearing three hairs.

The larger cyclops, Plate VIII., combined the following characteristics: the antennæ contained eleven segments; the rami of the swimming feet were three-jointed with an adult armature, as follows:

First Foot.		Second Foot.	
Outer Ramus.	Inner Ramus.	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
3 inner setæ,	3 inner setæ,	4 inner setæ,	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	Inner Ramus.	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	2 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta?
4 inner setæ,	3 inner setæ,	4 inner setæ,	2 inner setæ.

The rudimentary fifth foot was two-jointed with an almost square basal segment, bearing an immature seta externally, and a distal segment, longer than broad, armed with two immature spines and a median seta.

The study of a large number of these forms showed that both forms were always found together; that they always accompanied *C. signatus*, var. *coronatus*, having seventeen antennal segments; that in the absence of strong color markings and hairs they gave evidence of being young; and that they were, as a rule, sexually immature, although the larger cyclops occasionally contained ova. I became convinced of their larval character, as well as of their relationship to *C. signatus*, and determined to test the correctness of the conclusion by breeding experiments. Accordingly, a few fertile females with appended embryos, were isolated in a carefully prepared aquarium. At the end of two months this aquarium was examined for the desired forms, when nu-

merous individuals in both stages were found to be present. Later, one cyclops with eleven antennal segments was found with numerous large ova in the ovaries.

The rearing of the young from the adults proves conclusively that the forms in question are transitional stages in the life history of a form with seventeen segments in the antennæ. The experiments show also that *C. signatus* may become sexually mature in the larval state.

That this sexually mature young was a true larva, and not a form in which the growth of only the antennæ had been retarded, is indicated by the relatively small size of the individual when compared with the adults, by the incomplete number of segments seen in the antennæ, and by the transparency of the skin and the general absence of hairs and spines.

In the formation of the antennal segments there is a constant sequence. The nine-jointed antenna becomes transformed into the ten-jointed antenna by the division of the third segment from the base into two small ones, and the eleven-jointed condition arises from the ten-jointed, by the formation of a short second segment, the permanent second segment of the seventeen-jointed antenna. Occasionally the antennæ contain twelve segments, but intermediate conditions between the eleven and the seventeen-jointed stages are not frequent. In *Signatus*, prior to the completion of segmentation in the antennæ, two greatly elongated segments (the seventh and eighth in the eleven-jointed stage) break up simultaneously into the required number of small segments, four and three respectively, while the third segment breaks into two, thus providing seventeen segments, the number in the adult.

In a former paper, "Heterogeny and Variation in Some of the Copepoda of Long Island," I was of opinion that "some facts point to the probability that the Cold Spring Harbor forms . . . are morphologically undeveloped." I am now convinced that those cyclops combining nine, ten and eleven joints in the antennæ, with a two-jointed fifth foot, having a distal segment armed with three undeveloped hairs, are transitional stages in the life history of *C. signatus*, although the variety is less easily determined.

The study of various larval stages of *C. signatus* shows the rudi-

mentary fifth foot to be the most characteristic organ by which the species can be easily identified even in very early stages. Whether the hairs on the fifth foot are setose or spiny depends in large measure on the age of the individual, and is of little importance.

There are two generally recognized varieties of the species *C. signatus*, namely, var. *coronatus* (*C. fuscus* of Jurine) and var. *tenuicornis* or *annulicornis*, called *C. albidus* by Professor Marsh.

Both of these forms occur side by side and are regarded by some authors, Herrick and Brady, as transitional stages of one and the same form, while other authors regard them as distinct varieties.

I do not wish at this time to express an opinion on the relationship of *C. coronatus* and *C. tenuicornis*, but the correlations indicated in this paper for *C. signatus*, var. *coronatus*, can also be observed in the forms described as *C. signatus*, var. *tenuicornis*, in which the hairs on the inner margins of the stylets are lacking, as well as the serrations in the hyaline plate in the sixteenth and seventeenth antennal segments; and in which the second segment of the antennule, as well as the basal segment of the rudimentary fifth foot, are relatively long, while in *coronatus* they are conspicuously short.

The armature of the fourth pair of swimming feet, often shown in keys to the *Cyclops*, is by itself of little value as a means of identification, since several distinct species agree in the armature of this appendage, although they have no agreement in any of the other swimming feet, nor in the larger outlines of the body. It is to be regretted that fuller details of the *Cyclops* are not given as a means of securing a rapid acquaintance with common forms, and with their later transitional stages, which occur in great abundance side by side with the adults, and, like the adults, are sometimes found with eggs.

COLD SPRING HARBOR,
LONG ISLAND, N. Y., July, 1905.

PLATE VII.

C. signatus, coronatus.

Leitz camera drawings. Reduced one half.

FIG. 1. An outline showing the proportions of the thorax, abdomen, and antennæ when there are but ten segments in the antennæ. 3×3.

The abdomen has but three undifferentiated segments, to the last of which are attached the characteristic caudal stylets.

The antennæ reach to the posterior edge of the second thoracic segment, and contain ten segments each. Only the right antennule is represented.

FIG. 2. The external ramus of the fourth swimming feet. All the rami of the swimming feet are two-jointed. The proximal seta of the inner margin is conspicuously separated from the remaining setæ, which become the inner armature of the terminal segment after the formation of the third joint. 3×7.

FIG. 3. The rudimentary fifth foot is characteristic of the species *C. signatus*. The hairs are neither setose nor spiny. 3×7.

The internal fringe of hairs on the caudal stylets, the short, broad, basal segment of the fifth foot, and the short second joint of the antennule indicate that this is the larval form of *C. signatus coronatus*.

FIG. 4. An antenna with nine segments.

FIG. 5. An antenna showing the third segment dividing to form the tenth segment.

FIG. 6. An antenna with ten segments fully formed.

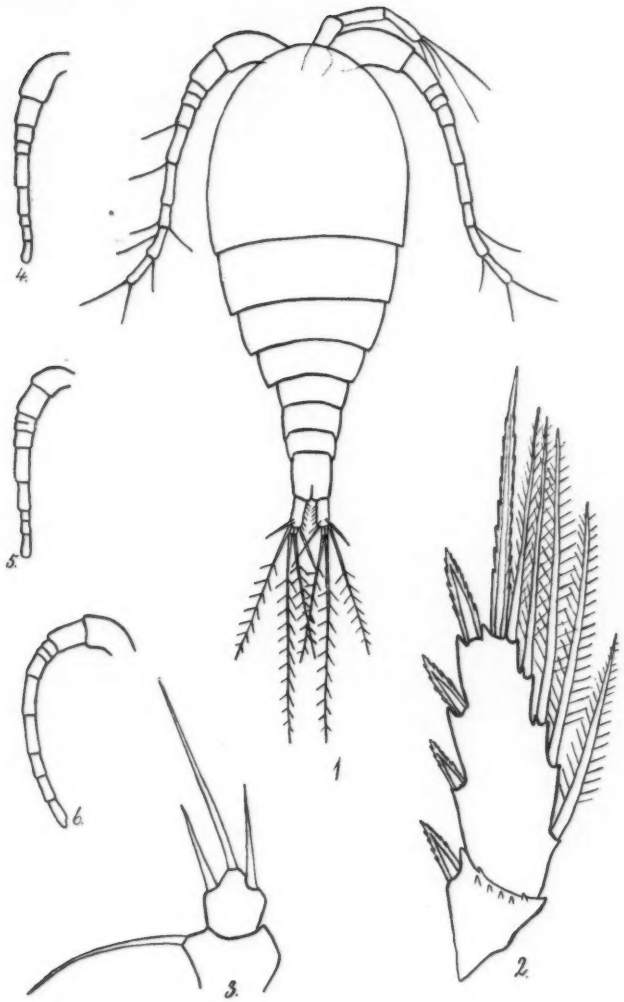
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PLATE VIII.

C. signatus, coronatus.

Leitz camera drawings. Reduced one half.

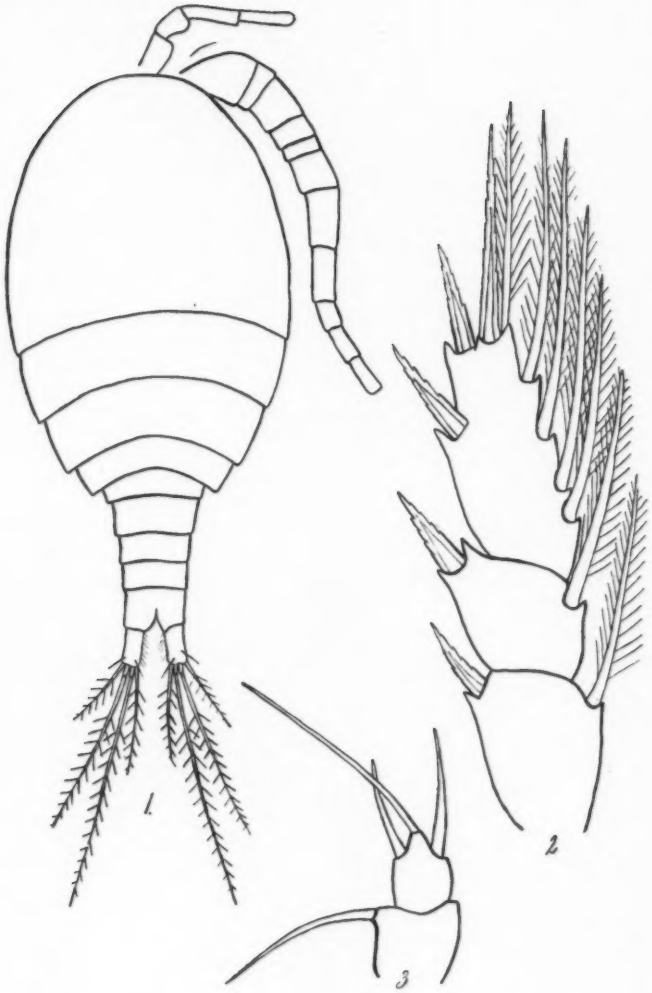
FIG. 1. An outline showing the proportions of the thorax, abdomen and antennæ when the antennæ contain eleven segments. 3 × 3.

The abdomen has acquired four segments in which there is still little differentiation. The caudal stylets remain unchanged.

The antennæ have acquired a new segment, the small second segment from the base of the antenna, making eleven in all. Only the right antenna is represented.

FIG. 2. The external ramus of the fourth swimming feet. All the rami of the swimming feet are three-jointed, and in their armature, have reached the condition of the adult. 3 × 7.

FIG. 3. The rudimentary fifth foot remains practically unchanged. 3 × 7.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THE MALE AND FEMALE EGGS OF PHYLLOXERANS OF THE HICKORIES.

T. H. MORGAN.

The predetermination of sex in the egg has been demonstrated only where small male and large female eggs occur, and it is a very striking fact, so far overlooked I believe, that in these cases the difference in the male and female eggs is connected with the development of degenerate males. In *Dinophilus apatris* the male is smaller than the female, is degenerate, and the sexual organs appear to be precociously developed. In *Hydatina senta* the male is smaller than the female and degenerate. In the *Phylloxera* of the grape and of the hickories the male is small, wingless, without digestive tract, and the sexual organs, as I shall show for one species at least, develop very early, so that the spermatozoa are fully formed before the male leaves the egg.

In *Dionphilus* it is believed that both the male and the female eggs are fertilized, but in *Hydatina* and in the phylloxerans the male and the female eggs are not fertilized. Sex in these cases is, therefore, determined independently of fertilization and pre-exists in the egg. In cases of this sort it would obviously be of great interest to discover what conditions determine that some eggs become males and others females. Is the difference, for example, connected with a visible difference of the nucleus, or of the cytoplasm? This question I believe I am able to answer, but the deeper-lying problem as to the causes that lead to the difference observable in the egg I have not fathomed.

The material for study was collected from the galls of the hickories in the spring and summer of last year, and included five or six species, of which three only will be mentioned here. The typical life history of these phylloxerans is the following: The fertilized egg, attached to the bark, hatches in the spring producing the stem-mother, who migrates to the young leaves and attaches herself to one spot on the under surface of the leaf, which becomes the center for the formation of a gall within which she becomes enclosed. She lays a large number of eggs inside

the gall that are all of one size, and give rise to the individuals of the second generation, which, developing wings in most species, ultimately leave the gall. These migrants contain two kinds of eggs, larger eggs that give rise to females, and smaller eggs that give rise to males. The eggs are deposited upon the bark

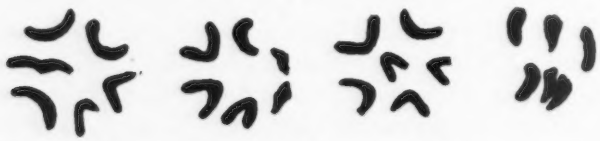


FIG. 1. *Phylloxera globosum.* Polar spindles of male and female eggs. The first figure (to the left) is from a large female egg; the second, from a small male egg; the third and fourth figures are from eggs whose size was not determined.

of the hickory. From them the male and female individuals are hatched. These soon pair and the female lays her single, large, winter egg on the bark of the tree. This egg hatches in the following spring, and produces the stem-mother.

A few species have a somewhat different cycle, and in one of them the male and the female eggs are laid within the gall itself. Owing to this condition a large number of the eggs in all stages of development can readily be collected. I have been fortunate enough to obtain a species of this sort, viz., *Phylloxera* sp.? and have obtained an abundance of developing male and female eggs. The life history of a species of this sort according to Pergande is as follows: The stem-mother lays eggs that give rise to a generation of wingless forms corresponding to the winged migrants of other species. These wingless individuals contain large and small eggs which they deposit within the gall. From these eggs the minute males and females emerge. In a few cases a winged individual — a migrant — is found with the wingless individuals. It seems probable that the wingless condition is the secondary one, hence the occasional appearance of winged forms.

The Male and the Female Eggs of the Migrants. — The eggs are mature in the migrant before it leaves the gall, and the polar spindle is present. It is a difficult matter to find the spindle, and the chance is also small of getting one cut parallel to its equatorial plate. Nevertheless, I have found a fair num-

ber of such cases in which the number of the chromosomes could be counted with perfect accuracy. In *Phylloxera globosum* there are only six chromosomes in the polar spindle (Fig. 1), which makes it a very favorable object for study. In all eggs in which the chromosomes could be counted the same number was found,

FIG. 2. *Phylloxera* sp.? Left hand figure is a spermatogonial equatorial plate. Right hand figure is from a somatic cell of a male embryo.

and this is true both for male and for female eggs. In this respect there seems to be no difference between the two kinds of eggs.

In *Phylloxera* sp.? the number of chromosomes in the male and female eggs seems to be twelve (Fig. 2). It is more difficult to count so many chromosomes with accuracy, at least in the eggs that I have so far seen; but as the clearest cases observed showed twelve chromosomes, and as in other eggs eleven, or twelve, or thirteen seemed to be present, and since the num-

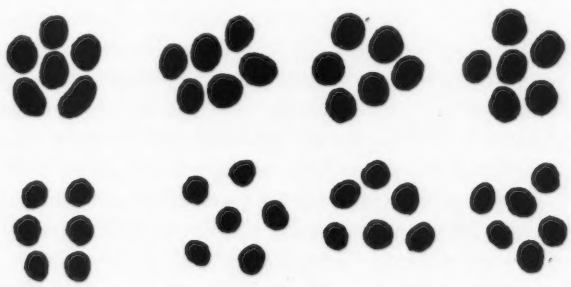


FIG. 3. *Phylloxera* sp.? Spermatocyte divisions. Equatorial plates. Upper row, first spermatocyte divisions; lower row, second divisions.

ber of chromosomes in the spermatocytes is definitely six (Fig. 3), there can be little doubt that the number in the polar spindle is twelve. In *Phylloxera caryæ-globuli* there are twenty-

two chromosomes present of very different sizes, as seen in the figures (Fig. 4).

In passing, it is worth while calling attention to the very different number of chromosomes found in these species of the same genus ; species in fact, that are so similar that they can only be distinguished with great difficulty. It does not seem probable

FIG. 4. *Phylloxera caryae-globuli.* Polar spindle-equatorial plate of egg of migrants.

in the light of cases such as these that the absolute number of the chromosomes can be a matter of any special significance. If the chromosomes are all composed of the same identical substance it is difficult to account for their constancy in number and sizes in each species. If the chromosomes are different in composition, as the conditions just mentioned would seem to indicate, the differences can scarcely be associated with differences in the structures of the body, since closely similar individuals are produced in one case with six chromosomes and in the other with twenty-two chromosomes. This question is one of the most puzzling problems in the whole range of cytology at the present time, and it would be unwise to draw any conclusion from the meagre facts known to us. I wish only to indicate as a possible view that the chromosomes may be different chemically from each other, and yet at the same time this difference may have no connection with differences in the form of the body.

Further History of the Chromosomes. — Starting with the stem-mother the history of the chromosomes through the life cycle is as follows : The number of chromosomes present in the polar spindle of the eggs laid by the stem-mother is the somatic or whole number. The number in the somatic cells of the embryo that develops from this egg is also the whole number. The number in the polar spindle of the male and the female eggs is,

as has been stated above, the whole number; and this is true for the somatic cells of both male and female embryos. In the spermatogonial cells the whole number of chromosomes is present, but in the two following spermatocyte divisions the half, or reduced number, occurs. I have seen the spindle in one winter egg, which seems to have only the half-number of chromosomes. It is evident, therefore, that the complete number of chromosomes is characteristic both for somatic and germinal cells throughout the life cycle, except for the winter egg and the spermatocytes, where the reduced number occurs preparatory to fertilization. After fertilization the number of the chromosomes would be the same as the whole number. Thus it is evident that there is no reduction in the number of the chromosomes in the parthenogenetic egg, but there is in the winter egg. The former is not, the latter is, fertilized. The results for *Phylloxera* are in these regards parallel to those that Stevens has recently obtained in the aphids.

The Cytoplasm of the Male and Female Eggs. — Since no discernible difference was detected between the chromosomes of the small male and the larger female eggs, I examined with some care the cytoplasm of these two kinds of eggs. In the male eggs there is less yolk and the center of the eggs is occupied by a clear mass of cytoplasm, while in the female egg there is more yolk and no central cytoplasm. I have not observed any other differences, and even those just noted can not be made out with certainty for all eggs, but represent the extremes of the series. Double and triple stains gave no differences that I could detect. In the ovaries the eggs showed no differences. In *Dinophilus*, according to Richard Hertwig, the large female eggs are formed by the union of several cells. It would be difficult to detect such a difference if it existed in the case of *Phylloxera*, since the ovarian eggs appear to be fused together at their inner ends, and each egg as it leaves the ovary remains attached by a cord of protoplasm to the fused center of the egg-mass.

There are striking differences in the development of the male and the female embryos, and these I have followed in some detail, but can not present the results at this time. One important difference must however be alluded to, namely, the precocious

development of the relatively enormous reproductive organs of the male. The testis is present as a large mass of cells at the time when the blastoderm is first laid down, and the spermatogonial divisions occur at this time. The two spermatocyte divisions occur when the embryonic plate is forming, and before the fibers appear in the nervous system. The size of the mass of spermatocyte cells is very large compared to the rest of the embryo, and it fills the greater part of one half of the egg. The spermatozoa are fully formed at the time when the embryo is developed.

The precocious development of the large testicular mass in the male egg suggests that a preëxisting mass of cytoplasm from which the testis develops may be present in the egg. The central mass of cytoplasm might supply such material, but I have not been able to make out any connection between the two. The result is due rather to the more rapid development of certain embryonic cells than to the presence of any large cytoplasmic mass of preformed material. The question arises whether we are to regard the large eggs that produce the females as doing so simply because they are large, or are they large because they are already female eggs? The latter alternative seems the more probable. In the light of certain recent experimental results, more especially those of Driesch and of Godlewski it seems highly probable that the *early* development of the embryo is due almost entirely to cytoplasmic influences. If this is true also in the case of the eggs of *Phylloxera*, then I think we may safely ascribe the difference in the size of the male and female eggs to the difference in the kinds of cytoplasm that are present when the egg is fully formed, so that the immediate determination of the sex is a cytoplasmic phenomenon. Whether this cytoplasmic difference can be traced to a preëxisting cytoplasmic basis, or to nuclear influence, or to the influence of external conditions is quite unknown, but in the absence of any nuclear differences it seem questionable whether we should assume that such exists.

THE ORGANIZATION AND EARLY DEVELOPMENT OF THE EGG OF CLAVA LEPTOSTYLA AG.

CHAS. W. HARGITT.[1]

A recent paper by Harm ('02), on the development of *Clava squamata* describes several features so unlike conditions which I have found in the American species, that it has seemed worth while to study anew, and in some detail, the entire life history, including also some interesting peculiarities of the organization of the egg not hitherto given any degree of attention among cœlenterates.

The general facts concerning the morphology, habits, etc., of *Clava leptostyla* Ag. were described long ago by Professor Agassiz ('62) with sufficient fulness to obviate the necessity of any considerable details as to these points. Allman ('71) has likewise given a somewhat similar account of *C. squamata*, though lacking in embryological details.

The following account is based upon studies carried on at Woods Holl during some two summers, chiefly upon living material, though for the details of cytology specimens were killed by several standard methods, among which were corrosive-acetic acid, picro-acetic, Carnoy's solution, Petrunkevitch solution, and corrosive formalin. Upon the whole, the first two of the reagents named gave by far the better fixation. As will be noted in another connection, for certain cytological results the picro-acetic solution was exceptionally good.

The most common habitat of the species in this vicinity was found to be the fronds of the common rock-weed, *Fucus nodosus*. Occasionally I have taken it from the piles of docks, or similar situations. It occurs most frequently in shallower harbors, or tide-pools, and is frequently found at low tide quite exposed, except as the moist vegetation may afford a measure of protection, lying thus for hours subject to the desiccating influences of sun and air, and apparently without serious injury. Of course, this

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

vicissitude is common to many organisms of similar habitat, yet few are so little protected by some skeletal feature as is this wholly naked hydroid. The breeding season at Woods Holl is apparently during June and early July, though colonies may be found in seemingly thriving conditions both earlier and later. Whether *Clava* like *Pennaria*, *Eudendrium* and others, has its phases of decline and recovery I have not been able to determine from actual observation, though the fact that at recurring intervals it appears in the same locations would seem to support this view.

Origin and Growth of the Eggs.

Weismann ('83) was the first to give any direct attention to this problem in *Clava*. According to him the ova arise in the entoderm in close relation to the supporting layer, though suggesting that perhaps the most primitive stages may have an ectodermal origin.

On this point Harm ('02) is quite specific in claiming their origin in the ectoderm, and their later migration into the entoderm, where in the region of the gonophores they become definitely differentiated and complete their development. This author even goes so far as to suggest that they may be distinguished in the planula as primitive germ cells, — "urkeimzellen" (p. 47, Fig. 48).

Of course, I am not prepared to discuss the matter so far as it relates to *C. squamata*, not having seen this species, but so far as it concerns *C. leptostyla* I have no hesitation in saying that eggs probably never arise in the ectoderm, but always in the entoderm of the peduncle of the gonophore, or in that of the polyp very near the base of the gonophore. In thousands of sections studied, both by myself and by several of my students, there has been no exception to this statement of fact. *Clava*, like other hydroids, has its breeding season, during which the germ-cells are extremely abundant, and at other times these cells are either entirely absent or very scarce. Again they do not appear except in direct relation to the forming gonophores or in that immediate region. In fact in *Clava leptostyla* the morphology of the gonophores and their development as dense, bud-like clusters from a single peduncle to which they are attached by narrow pedicels, make it

almost certain that the vast majority of all egg-cells arise in the gonophores themselves as, of course, is the case in almost all free gonophores, or medusæ, and as is invariably the case with the spermatozoa.

Therefore, while it may not be impossible that "urkeimzellen" should perhaps exist in undifferentiated stages, still the probability is so extremely remote as to render doubtful to a degree any but the most thoroughly substantiated claims.

It may be stated in passing, that the gonophores of *C. leptostyla* are extremely degenerate, hardly more, indeed, than sporosacs, yet it is possible to distinguish rudiments of medusoid structures. They originate as buds of the hydroid, involving both ectoderm and entoderm, and also supporting layer. Occasionally this lamella seems to partially disintegrate at the terminal portion during the outgrowth of the peduncle. The gonophores remain entirely closed except at the time of rupture by the escaping planula. From the primary peduncle secondary pedicels arise, forming a racemose-like cluster, within each of which from one to four eggs may develop, though the usual number is two or three.

There seems to be considerable variation in the size of the eggs in various specimens and in the eggs of various gonophores of the same specimen. On this point it had occurred to me that perhaps the number developing in a given gonophore might naturally have some influence, but after comparing a considerable number, whether growing singly or in clusters I have not been able to convince myself that such is the case. I am rather inclined to believe that more depends upon the start a given egg may get in growth, and perhaps the state of nutrition in which the given specimen may be at the time, than any other factors.

As a rule eggs which grow singly at the distal end of a gonophore are more nearly spherical, and in consequence I find the cleavage of such eggs much more regular and symmetrical than in cases where two or more are found in the same gonophore and approach maturity at about the same time. This point may be considered in more detail in a later connection under the subject of cleavage.

Growth takes place, as in most hydroids, quite rapidly. With

the origin of the gonophores the eggs appear in considerable numbers in the entoderm of the peduncle, as previously stated, and with the later budding of the gonophore bodies either migrate into them, or as seems to be the case in many instances, originate directly from the entoderm of the spadix or lateral walls of the gonophore. At first they have the characteristic aspects of ordinary ovocytes, namely, a very large germinal vesicle, with characteristic chromatin network, a comparatively small proportion of cytoplasm, which is more or less homogeneous in texture, and staining quite uniformly with any of the ordinary plasma stains. Evidences of growth are first indicated by the rapid increase in the mass of the cytoplasm, while that of the nucleus for a time remains apparently unchanged, though later also increasing in mass likewise, though to a much less degree. Nutrition of the ova is at first, indeed throughout so far as I can determine, by direct absorption from the entoderm of the spadix, or to a less degree also from the entoderm of the lateral walls of the gonophore. I find no evidence of the absorption of supernumerary ovocytes involved in the matter of nutrition, and in this respect *C. leptostyla* appears to differ somewhat from *C. squamata*. According to Harm there would seem to be involved both these processes. He says that by the direct assimilation of yolk-like granules from the entoderm cells of the hydranth, and by osmosis from the walls of the gonophore the egg is nourished, and that furthermore, the youngish egg-cells are also nourished by the absorption of ovocytes (*op. cit.*, pp. 11, 12).

The presence of the yolk-like particles to which he refers I have also recognized in the entoderm of the hydranth body at this period. They resemble in all essentials the pigmented yolk granules later found in the fully grown egg, but I have found no evidence that they are ever directly absorbed by the young egg. On the other hand there is ample evidence to the effect that they are gradually broken down and probably liquefied, in which condition they may be easily transferred to the gonophores and absorbed by the young eggs. In an earlier paper ('04) I have directed attention to similar phenomena in the growth of the eggs of Pachycordyle, and it undoubtedly occurs in many others.

Hermaphroditism.

An interesting feature in the reproduction of this hydroid is the fact that occasionally individuals, and perhaps colonies, are found in which gonophores contain both eggs and spermatozoa. Figs. 1, 2; Fig. 5, Pl. IX., show various phases of this somewhat anomalous condition. As will be observed, the elements are in various stages of development, some of the eggs well along toward full growth, and spermatozoa likewise well advanced. In most cases the condition shown at Fig. 5, Pl. IX., was the prevalent one, namely, where one half of the gonophore bore sperms and the other half an egg. In a few cases, however, a well developed egg was found on each side nearly or quite surrounded by sperm-cells.

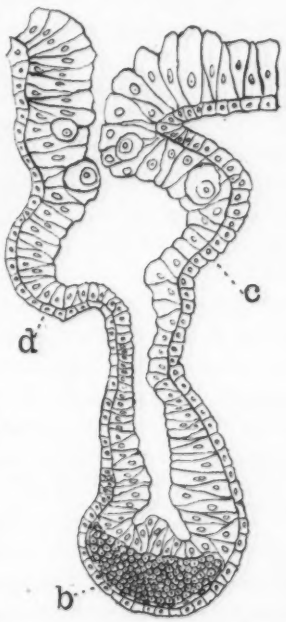


Fig. 1. Longitudinal section through young gonophore, showing at *b* the development of spermary, at *c* and *d* mixed gonads.

Of course, hermaphroditism in itself is nothing strange among animals, whether high or low. Even among hydroids it is quite familiar in the common *Hydra*, though here it is not common to find both organs in active function at the same time on any given individual. I have also found a similar condition in *Hydractinia*, and Bunting ('94), has likewise figured a single case though without giving any details concerning it. In the whole of his extended researches on the "Origin of Sex Cells in Hydromedusæ" Weismann makes no mention, so far as I have observed, concerning hermaphroditism. It would seem somewhat remarkable that he should not have observed some indications of such a condition if it were at all common. Indeed, though having found repeated cases of it in *Clava*, I am disposed to consider it as a rather rare phenomenon in this group.

One other feature in connection with the subject must be noted, namely, that among the several cases, the ova were found in every case in distinctly male gonophores. Among hundreds of female gonophores examined there was not the slightest evidence of male elements among them. Figs. 1 and 2 show sections through two hermaphroditic gonophores. At *b* Fig. 1 is developing a

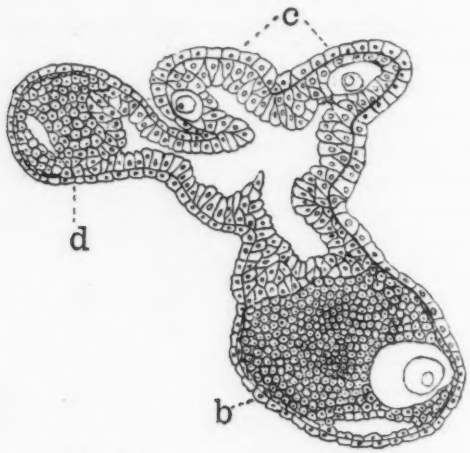


FIG. 2. Cross-section of hydranth showing development of gonophores. *b*, hermaphrodite gonophore; *c*, early stages of same; *d*, spermary.

typical male gonad, while at *c* and *d* are shown what are evidently destined to be mixed gonads. Similar conditions are also shown in Fig. 2, which is a camera sketch of a section across the entire hydranth and gonophores.

It has long been known that among actinians a form of hermaphroditism, involving successive sexual rhythms, known as protandry or protogyny, occurs. Duerdon ('04), in his studies of West Indian corals has found warrant for believing that protogyny is the predominant condition, since "spermaries have never been found alone, but always associated with large numbers of ova; on the other hand, many polyps have been found with ova alone, often few in number, as if sexual maturity were but beginning." At the same time he quotes from Mr. Stanley Gardiner, who having studied a large number of developmental stages in *Flabel-*

lum rubrum, has been able to show that in this form spermaries arise first on the mesenteries and that ova appear later, when the production of sperm acini ceases. "The ova grow enormously with the final result that the mass becomes entirely female, consisting usually of two or three large ova, flattened on their sides against one another and occupying the whole area of the former testes."

Hermaphroditism is also known among Scyphomedusæ, Wright having described in some detail the chief features in the case of *Chrysaora hyoscella*. He says, "Large individuals are hermaphroditic, but smaller ones are found which are unisexual, the male or female element being suppressed. Small Chrysaoras (about four inches in diameter), have no ovarian bands in their pouches, which only contain masses of the grape-like bodies (testes), and tentacles before mentioned."

This would seem to imply that here again we have protandrous hermaphroditism, the spermaries developing first, and later the ovaries. Incidentally, it may be observed in passing, that this author in his account of the development of the eggs of this medusa was much impressed with the absence of any germinal vesicle. "The ova of *Chrysaora hyoscella* do not present, at any stage, a trace of the germinal vesicle, — objects which are so readily detected in the ova of other polypoid Zoophytes."

Haeckel ('79), who has also studied the development of the medusa, was able to confirm Wright's account as to hermaphroditism. "In Uebereinstimmung mit letzteren habe ich gefunden, dass junge Chrysaoren rein männlich vorkommen, solche mittleren Alters meisters Hermaphroditen sind, und endlich ganz alte Thiere meisters nur weiblich sind, oft noch mit Ueberresten männlicher Organe."

So far as I have been able to discern in the case of *Clava*, there is no evidence to indicate the operation of either of these oscillating phases of sexualism. I have specimens taken at all times of the breeding season and have found no tendency toward the one or the other. I am rather disposed to regard it as an expression of a mutative impulse, in response to which in other forms, such as those already cited, these interesting features became established.

Organization of the Egg.

It has long been known that simple though the egg may be it must, nevertheless, be regarded as potentially highly complex. Concerning the early views of His, Whitman, Flemming and other earlier investigators, no attempt will be made here to give special citations. The later experiments of Roux, on the development of the frog's egg, supplemented by similar experiments by Driesch '95, served to emphasize still further the general view here stated.

The still later investigations of Wilson ('03, '04), and Conklin ('05[1], '05[2], '05[3]), have given a new impulse to researches along this line, and have clarified and measurably harmonized the conflicting views of earlier observers.

So far as I am aware, no one has shown any evidence of anything of a similar character in the organization of the eggs of cœlenterates. In commenting on the account given by the present writer of the cleavage of the egg of *Pennaria* Conklin ('05[3]), has suggested the probability that predetermining factors must be present in some form. "Even in such eggs as that of *Pennaria* it is certain there must be determining factors somewhere, if not in the cytoplasm then in the nucleus, which determines that the egg shall develop into a pennaria rather than into some other animal; it is further evident that these determining factors must be present in the cytoplasm at a relatively early stage, if not in the very beginning of development" (p. 215).

Hence in my studies on hydroid eggs rather particular attention was given to this point with the hope that some evidence might be found for or against the views in question. In my work on *Pennaria* ('04[3]), while no direct evidence of cytoplasmic differentiation was found in the living egg, there was found in some cases after fixation by certain reagents, more particularly the picro-sulfuric solutions, what seemed to be a stratified, or concentric arrangement of the cytoplasm. At first I was inclined to regard this as probably significant of such locally differentiated matter as might go to form the ectoderm. But since this condition was not found to be constant it was regarded as probably an artifact, due to the action of the reagent. A recent examination of my earlier preparations has not suggested any change of

this opinion. Indeed, as pointed out before, the extremely erratic and anomalous behavior of these eggs during cleavage would seem to render extremely difficult, if not impossible, any predetermining factors in either cytoplasm or nucleus, whose influence could be maintained during the varying and indeterminate process of development. While we may readily admit that, as Conklin has suggested, there must be factors present which determine that the egg shall develop into a *Pennaria*, still this does not compel the conclusion that therefore they must be definitely localized. It is simply a matter of heredity; and if it be true as generally contended that this is a problem of chromosomes; and if, as I have shown in the case of both *Pennaria* and *Eudendrium*, the chromatin may be more or less dispersed throughout the entire cytoplasm during maturation and early cleavage, then definite localization in one or the other is not involved in Conklin's sense of the term. However, it must be regarded as a question of fact, and so far as evidence exists in the present case it would seem to be opposed to the theory of localization.

During June and July of the past summer I carefully studied the living eggs of *Clava*, and with this point still clearly before me. As pointed out in an earlier section, the eggs originate in the entoderm of the gonophore, and grow by direct nutrition derived from the cells of that tissue. A study of the eggs in various stages of growth revealed the appearance at a certain stage of development of a delicate, bluish pigment, which gradually accumulated in amount as the eggs approached maturity. This was carefully observed in the living specimens and has since been studied in sections after a variety of fixations. At first the pigment makes its appearance in the immediate region of the nucleus, about the time that body takes its place at the outer periphery of the egg. This is shown in Fig. 1, Plate I. From the nuclear region the pigment extends as a crescentic disc outward, forming later a peripheral zone which finally extends over the entire egg, though this rarely occurs until cleavage has made some progress.

An interesting fact observed in this connection was that the amount, or at any rate the color-intensity, of the pigment differed considerably in different specimens. This was particularly the

case in colonies which had been kept in the aquarium for a few days. In these the pigmentation was appreciably less intense than in specimens freshly collected. There were, however, notable exceptions in this respect among various specimens under natural conditions. The same has been observed in *Pennaria*, and is, indeed, a fact more or less well known in many animals. Conklin ('05[1], p. 13) has cited similar cases among ascidians observed by VanBeneden and Julin; in some species two very differently colored eggs being produced, one yellow, the other gray. Both are said to develop normally and in the same manner, giving rise to larvæ whose entoderm cells are of the same respective colors.

In the case of *Clava* there is no such distinction as this, though the presence of more or less pigment has apparently no effect upon the normal development of the embryo. In another point there is also some measure of similarity, namely, in that as development proceeds, the pigment which was at first distinctively peripheral in position, seems later to become transferred to the entoderm of the larva. This, however, as will be seen later, is due not to any shifting of this matter from one region to another, but simply its resorption by the more rapidly developing ectoderm, while as yet the entoderm is only partially differentiated.

Whether this pigmentary zone formed a definite germinal area, and its gradual development was an expression of the differentiation of this area, or whether it might not be simply the results of cytoplasmic metabolism associated with the formation of yolk substance, or whether it might not, perhaps, be in some way associated with the phenomena of maturation, seemed for a time somewhat uncertain. A study of the matter more critically in its cytological aspects soon sufficed to discredit the last alternative, namely, that it was in any way associated with maturation. Furthermore, the appearance of the pigment was too early in the history of the egg to involve the operation of any maturation phenomena.

Again, several series of facts conspired to discredit the probability of the first alternative, namely, that it was in any sense a differentiation of germinal substance. Of these the following may

be particularly mentioned: (1) The entire absence of any correlation between the pigmentary zone and the course of cleavage; (2) the continuous development of pigment, even after the completion of cleavage, and after the ectoderm has been clearly established; (3) the staining reactions of the granules after hardening is comparable, point by point, with that of yolk granules; (4) finally, the granules are gradually reduced and resorbed with the growth of the embryo. It is in this way that their disappearance from the ectoderm above referred to is to be explained.

I think that we may therefore conclude that, at least so far as the problem is concerned with *Clava*, its solution is unquestionably in the negative. If further warrant be needed for this view I believe it will be found in the later history of these pigmentary granules as given in the following account.

Origin and Growth of Pigmentary Granules.

As already noted the origin of these pigmentary granules is in the immediate region of the nucleus, and about the time this body reaches the outer periphery of the egg. At first they are of extremely small size, about 0.5 micra in diameter, later growing and reaching in some cases a diameter of 3 to 3.5 micra. For some time they were entirely overlooked in prepared material, owing to improper fixation. Only after fixation with picro-acetic (or to less degree with Petrunkevitch) solutions and staining with iron-hæmatoxylin on the slide were they adequately differentiated so as to be readily studied. Wilson gives a similar account of this technic in reference to the eggs of *Dentalium* (cf. *Jr. Exp. Zool.*, Vol. I., p. 9, Figs. 10–13, explanations).

As the granules continue to grow larger there may be distinguished within their substance what appear to be vacuoles, usually a single one within each granule, occupying an eccentric position. In some respects they exhibit nucleolar-like features, especially in their staining reactions and in their vacuolation. Montgomery ('98) has made similar observations on nemerteans and believes they indicate some sort of genetic relationships between nucleoli, yolk balls and granules. My own observations have not seemed to confirm this last point, though in the case of *Hydra* there are not lacking evidences which I think would give

strong confirmation of Montgomery's views. This point may have further consideration in connection with observations upon the history of the nucleolus.

Concerning the real nature of these granules there arises the query, are they katabolic products, associated with some vital wastes incident to the cytoplasmic activities of growth, or are they not rather anabolic in character, highly nutritive proteid bodies, analogous to yolk matter and of similar import? The latter is by far the more probable view, though there are points of difference as compared with the usual formation of such nutritive matters. For example, in most hydroid eggs which have come under my observation the development of yolk granules has no appreciable relations to nuclear influence, and seems to be for the most part developed and deposited chiefly at the vegetal pole of the egg; while in the present case, as has been shown, they seem to arise and develop chiefly in the nuclear area and only at a late period are found at the vegetal pole. However, I am inclined to believe that this is not a serious difficulty. It will be observed that the vegetal pole lies in immediate contact with the spadix of the gonophore, and that the reception of nutritive matter by the growing egg is from this source. Of course, this nutritive matter is in the form of liquid, and so long as the egg is continuously receiving it in this way there is no occasion for further metabolism into the more solid reserve of yolk. The remoter animal pole where such reserve would first be needed begins the anabolic process first, and with the gradual suppression of the nutritive activities of the spadix the process of proteid anabolism would extend into that area.

According to Harm (*op. cit.*, p. 19), the development of this yolk matter in *C. squamata* presents some rather sharp contrasts as compared with the above. For example, instead of developing more or less gradually, and spreading from the nuclear region over the surface of the egg, he finds it arising somewhat suddenly and equally throughout the entire egg. "Bald nachdem die Eizellen Glockenkern erreicht haben, beginnt in ihnen die Dotterbildung, die an allen Stellen zu gleicher Zeit einsetz." He also finds that before the formation of yolk granules the eggs are reddish in color. "Während die lebende Eizelle vor der Dotterbil-

dung röthlich erscheint, zeigt nacht derselben eine blaugraue Fährbung." This point is confirmed in the case of *C. leptostyla*, in which essentially the same process takes place.

Perhaps a few words may be added as to the significance of the development of pigment in connection with these yolk granules. I have in recent papers ('04[1], '04[2]), submitted certain views as to this subject and it may suffice in general to refer to those discussions. In the former it was said that pigments in organisms might appear under three aspects: (1) Those directly serviceable to the organism, as in chlorophyll, hæmoglobin, etc.; (2) as waste products, which embrace probably the more numerous of organic pigments, such as guanin, melanin, etc.; (3) as reserve products, of which the lipochromes are typical. In all probability the various pigmentary matters found in eggs belong to the third of these classes. And here undoubtedly should be classed the pigmentary granules of *Clava*, and other similar pigments of hydroid ova. In the second paper attention was directed to a special case, that of *Pachycordyle*, already referred to, in which one may trace the various stages in the growth of the egg and the formation of the pigmentary bodies. Here as in *Clava* there can hardly be reasonable doubt that the process is a gradual and progressive anabolism, so far as the granules themselves are concerned, but it must still be a somewhat open question as to the exact relation of pigmentation thereto. May it not be probable that here, as in many of the more active phases of metabolism in which pigments are more or less evident expressions of excretory, or waste products, the pigment itself, though associated with anabolic activities, is an expression of the correlative process of catabolism? In other words, that even in those constructive processes involved in the storage of reserve matters, whether as proteids, fats, or whatever they be, there is involved the inseparable process of energy bestowed, and that as one of the signs of such energy its imprint is left in these pigmentary elements? Such I am inclined to believe is what actually happens. And as the nature of these processes differ more or less in various organisms so the pigmentary signs of waste will likewise differ. Hence the purple pigment of the eggs of *Clava*, the pinkish of *Pennaria*, the reddish of *Eudendrium*, etc.

Maturation.

Concerning this phase of development there is comparatively little to be said in the case of *Clava*. I have studied as critically as the nature of the egg would allow the behavior involved in maturation both in living and preserved material, and in a very large number of preparations, but with almost wholly negative results. That is, I have found the phenomena to be so obscured by the opacity of the cytoplasm, or by the pigment matter in the yolk, or as seems to me a still further probability, namely, the extremely fugitive character of the phenomena, as to render them indistinguishable. I have had occasion to emphasize this matter in several earlier papers dealing with the subject. The observations made upon the eggs of *Pennaria* have been duplicated, almost point by point in the present case. Of course, in the egg of *Clava* there is the added di culty that all the phenomena occur within the closed gonophore. In sections, however, this fact ought to offer no serious obstructions to their detection, yet the results, as in the former, are quite as uncertain and in most cases absolutely lacking.

In the case of *Clava squamata*, Harm (*op. cit.*, p. 23) describes the phenomena in some detail, and gives almost diagrammatic drawings of the several stages. However, as will be further shown in connection with the cleavage phases, there are so many points of difference between these species that the ova may perhaps belong to very different classes so far as their character and texture are concerned. From the fact that in several particulars I have been able to confirm the observations of Harm it does not seem probable that they are so greatly different as might be implied.

I shall briefly describe the principal features which it has been possible to certainly determine, leaving others open to further inquiry or study.

As pointed out in an earlier section, about the time the ova approach full size the nuclei are to be found close to the outer periphery as will be seen in several of the accompanying photographs. It will also be observed that the eggs occupy closely the entire space of the gonophore, and that, therefore, the nuclei in coming in contact with the gonophore wall become more or

less flattened. It is at this stage that the first indications of maturation becomes apparent, namely, the shrinking and gradual disappearance of the nuclear membrane. This is particularly well shown in Fig. 2, Pl. IX. About the same time, or in some cases slightly before, there is also an evident dissolution of the chromatin network, unaccompanied by any indications of chromosomes. In this respect these eggs closely resemble those of *Eudendrium* and *Pennaria*. The most painstaking attempts to differentiate these bodies by staining operations have as frequently failed. It seems to me we are forced to the conclusion that the appearance of dissolution is indeed a fact, and that at this stage there is a general dissipation of chromatin, and perhaps other nuclear matter, into the cytoplasm.

Behavior of the Nucleolus. — Usually at about this stage marked changes take place in the nucleolus. In several instances it has been found to migrate bodily from the germinal vesicle into the cytoplasm where it is gradually dissipated and probably assimilated. At this point I find my observations closely in accord with those of Harm (*op. cit.*, p. 24). "Wahrend also hier der Nucleolus in toto vor der Polkorperchenbildung aus dem Keimblaschen heraustritt, um vom Eidotter aufgenommen und resorbirt zu werden, verbleibt er in anderen Fallen in demselben und zerfallt dort in mehrere Kugelchen."

Just prior to this migration of the nucleolus into the cytoplasm it was found to show varying degrees of vacuolation, in advanced stages of which it was often seen to partially shrink and collapse, as if there had been a loss of nucleolar substance. Something of this may be observed in several of the photographs already referred to.

Conditions very similar to these I have elsewhere described in connection with other hydroid eggs (*op. cit.*, 04[4], p. 562). Montgomery has cited many similar features which had come under his own observations, as well as observations of a like character made by many others. Those who are particularly concerned will find his discussion exceedingly interesting and suggestive, as well as including a valuable summary of evidence bearing on this problem. I may say, however, in passing that I have failed to find any indications in the present case of the metamorphosis of

the vacuolated portions of the nucleolus into yolk granules, such as Montgomery has described. On the other hand the whole of the nucleolar substance seems to be directly dissipated throughout the cytoplasm and indistinguishably assimilated by it.

Polar Bodies. — With the phenomena already described there have been found in a few cases what seemed to be polar bodies. But it was impossible to distinguish the presence of any mitotic mechanism. In every case the nuclear matter was devoid of any trace of chromatin, or, as just suggested, mitotic figures. In a few cases the formation of these bodies was observed in living eggs, and these showed essentially the same features. The nuclear matter being in close contact with the gonophore wall, small rounded portions seemed separated from the larger part, and could be distinguished close under these retaining membranes for only a short time, when they gradually disappeared, apparently resorbed, as I have suggested in former studies upon *Eudendrium* and *Pennaria*. In this respect also my observations correspond with those of Harm, though I have in no case been able to confirm his account of the phenomena of mitosis. "Die Resorption der Polkörperchen durch die eizelle sehr schnell vor sich gehen, da ich dieselben zur Zeit, wenn der weibliche Pronucleus besteht, nicht mehr habe nachweisen können."

The results in the present case, as in those of *Eudendrium* and *Pennaria*, already referred to, as well as in certain others, serve to suggest the query whether, indeed, there may not be great variation as to phenomena of maturation, even perhaps to the extent of the suppression of the more conspicuous aspects associated with it in higher forms. It may be regarded as the wildest biological heresy to even remotely suggest that in some eggs these physical phenomena might be entirely absent, but such an impression has grown upon the present writer for some time and increases with each further case, such as that under consideration. If we may have normal nuclear division without mitosis in the early embryonic history, and this I believe to be fairly beyond doubt; and if we may have differentiation without cleavage, now likewise admittedly true; and if, furthermore, we may have prior to either of these phenomena in development the dissipation of both chromatin and other nuclear matter throughout the

cytoplasm, another fact of which I can no longer doubt, then it does not seem a far call to the assumption that the reduction phenomena of maturation may well be accomplished without any of the complex and spectacular processes of mitosis. Why should the egg go through with the physical process of extruding the polar globules if they are to be immediately resorbed by the cytoplasm ?

I do not overlook the circumstance that the opacity, and in the present instance pigmentation, of the egg may establish an obstacle so formidable as to render accurate observation extremely difficult, as I have previously admitted, still the fact remains that carefully preserved and stained sections of hundreds of eggs have failed to afford convincing evidence of the presence of these bodies in distinguishable form, except in doubtful cases already referred to. It would seem somewhat remarkable that there should be all the differential results of staining upon other cell features while it should be uniformly lacking in these, usually among the most readily demonstrated.

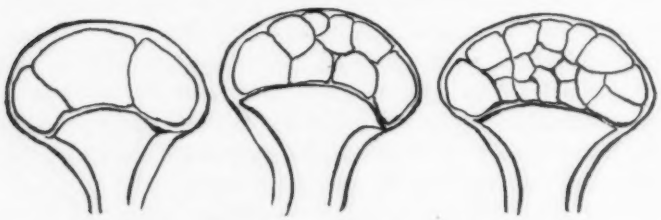
Yet another feature bearing on this point will be found in the details of cleavage, during which there is every appearance of spontaneous nuclear reorganization throughout large portions of the egg at almost the same time, the last detail of which may be followed. The facts will be described in a later section, though it seems well to call attention to their significance in relation to the matter under consideration.

Cleavage.

In a general way cleavage in these eggs corresponds with that of other hydroids having similar gonophores, such as *Tubularia*, *Hydractinia*, etc. The earlier work of Ciamician ('79), and Brauer ('91), on *Tubularia mesembryanthemum*, and the later work of Allen ('00), on *T. crocea*, afford good examples of the type of cleavage here referred to. In a recent paper ('04[4]), the present writer has briefly reviewed the results of Ciamician and Brauer, and it is unnecessary to discuss here in any detail these features. Suffice it to say that Brauer found what he regarded as two rather distinct types of cleavage. The first more or less regular and equal ; the second irregular and indefinite, involving

for a time an internal nuclear proliferation, followed later by the spontaneous division of the cytoplasm into a corresponding number of blastomeres. While my own observations (*op. cit.*) did not fully confirm those of Brauer they abundantly proved the general proposition that cleavage is not uniform, either in mode or progress. The work of Allen (*op. cit.*) showed even more conclusively the variation of this feature in *T. crocea.*

The same thing is true in the eggs of *C. leptostyla.* While there is here much more uniformity than in either of the former cases the range of variation is still very considerable, as a glance at the various figures will abundantly show.



FIGS. 3, 4, 5. Camera sketches of phases of cleavage, from life.

The account of Harm (*op. cit.*, p. 28) concerning cleavage in *C. squamata* is in very marked contrast in its general aspects. Both in his descriptions and figures there is a most striking regularity and definiteness in the cleavage. He very briefly refers to a condition which he considers exceptional and abnormal, but which unless I greatly mistake must be much more common than he is disposed to think. It is quite similar to a condition referred to by Brauer, and regarded by him as likewise exceptional, but which I have showed to be more or less common.

Again, Harm describes in some detail what he regards as a more or less definite rotation of the blastomeres during earlier cleavage, in some measure comparable with phenomena familiar in the eggs of annelids and molluscs. When attention is directed to the fact that in these hydroids the eggs are usually quite closely confined within the closed walls of the gonophore, and that, whether there be two or even more in a single gonad, they are always more or less flattened against each other or against

the spadix, and that the entire course of development takes place within these walls it is difficult to see how any considerable movement of the blastomeres upon each other can be possible. Or, furthermore, how it is possible to have anything like the regulation type of cleavage characteristic of the forms just referred to, unless, perchance, the gonophores of *C. squamata* differ very greatly from those of *C. leptostyla*, which hardly seems probable. At any rate, I have not found it possible to trace any close correspondence to such features in our species, as will be seen from the following account.

As stated in an earlier connection, the gonophores of *C. leptostyla* may contain only a single egg or as many as four, though the usual number is two or three. In many cases where but two

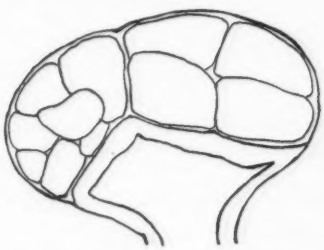


FIG. 6. Camera sketch of gonophore containing two eggs at slightly different stages.

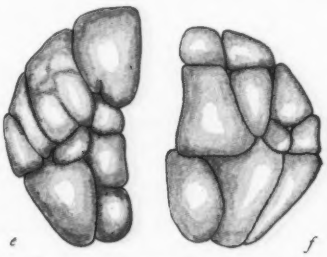


FIG. 7. Camera sketches of two eggs, *e* and *f*, as seen from side.

are found they will be upon opposite sides of the spadix, but frequently also supported side by side at the terminal portion. In either case the eggs are flattened very much on one or more surfaces, as shown in several of the figures (Fig. 7, *e* and *f*). In some cases they may even come to have a biscuit shape, or may be crescentic disks, as also shown in Figs. 3–5. In many cases as the eggs approach full growth there is a tendency to become more or less spherical, especially when occupying singly the terminal portion of the gonophore. Now, I have found that these various conditions have a more or less marked effect upon the mode of cleavage. Where the egg is spherical, for instance, cleavage is usually more or less symmetrical, as shown in Fig. 8. On the other hand, where the conditions maintain a continued

pressure the cleavage is very irregular and unequal, as shown in Figs. 6 and 7, drawn from life with the aid of the camera. In Figs. 10 to 18 are shown conditions found in sections of an egg,

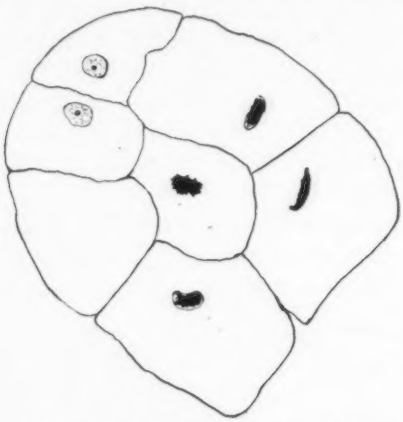


FIG. 8. Sketch of section of egg, showing nearly regular cleavage. In two cells are resting nuclei, and in others various phases of nuclear reorganization.

which are almost exactly equivalent to similar sections obtained in the study of *T. mesembryanthemum* ('04[4]).

I think it will be evident, from even a cursory study of the several figures and photographs, that the cleavage in this egg is, like that of *Pennaria*, more or less erratic and indeterminate, and conforms to none of the regular types.

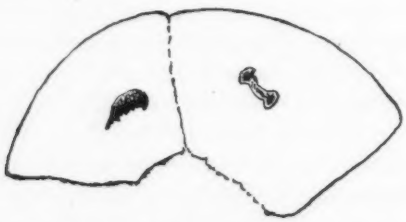
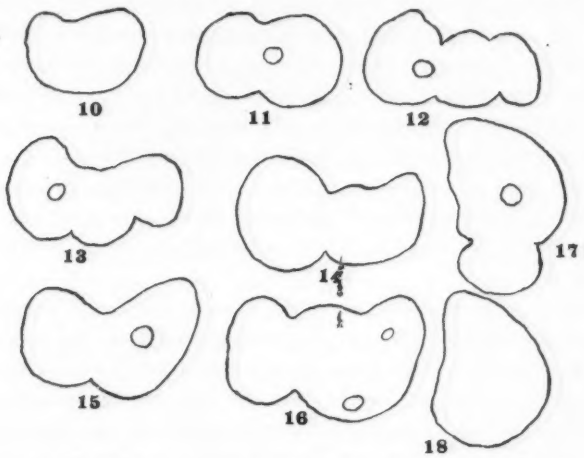


FIG. 9. Sketch of two cells of egg section, showing in one a dumb-bell shaped nucleus in amitotic division.

Another feature of cleavage remains to be considered, namely, one which involves chiefly, perhaps in some cases wholly, the

nuclei. In *Eudendrium* and in the species of *Tubularia* referred to above, it has been shown that in a considerable number of eggs there was formed by nuclear proliferation an evident syncytium, and that from this there was later a differentiation of the embryonic tissues without the process of ordinary cleavage. I have found something of the same kind in the case of *Clava*. Occasionally an egg was found among serial sections which showed no evidence of cytoplasmic cleavage, but where internal nuclear proliferation was clearly evident, and the specimens were



FIGS. 10–18. Outline sketches of sections of single egg, showing the various distribution of nuclei, shape of egg, etc.

numerous enough to enable one to definitely determine the phases of tissue differentiation and the formation of the embryo. In these cases were found the same evidences of the origin and organization of nuclei *de novo* which were found in *Eudendrium* and *Pennaria*. It was possible to trace almost every phase of this nuclear organization, from the appearance of the smallest particles of chromatin and their segregation into larger masses to the fully formed resting nucleus with its typical elements in normal relations and proportions.

Amitosis and Nuclear Organization. — During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has

been found. I have already directed attention to its entire absence during maturation also. Now it is interesting to find abundant mitoses during the later cleavage, and during the growth of the embryo. Is it not somewhat remarkable that among the eggs of a given gonophore cluster in various stages of cleavage and later embryonic development there should be found in certain cases abundant mitoses while in others their entire absence? This cannot be attributed, under such circumstances, to differences of fixation or other details of technique, for this has been identical throughout. To what, then, may it be attributed, or how explained? As already suggested in connection with the subject of maturation, it seems to me we are forced to assume the operation of some extremely obscure bio-chemical changes which neutralize alike both acid and basic stains, or that certain phases of the mitotic mechanism may be disguised or actually lacking.

In the light of cumulative evidences along this line I think one may safely assume that the second of these alternatives is the more probable, and the facts here submitted strongly support this conclusion.

During the earlier aspects of development the nuclear phenomena associated with these eggs are extremely indefinite and obscure. During maturation there seems to be an actual fragmentation and dissipation of the nuclear matter throughout the cytoplasm. The first signs of its reorganization appear in the segregation of chromatin-like masses during the early phases of cleavage, about which the cytoplasm becomes organized into cell-like masses, as suggested in an earlier connection. At this time these chromatin masses are extremely irregular in form and size, stain very densely, and appear as indefinite, flocculent patches occupying the center of cytoplasmic aggregates, or cells. They usually assume an elongate, or often dumb-bell shaped form and seem to divide amitotically, followed by the division of the cell. In Fig. 8 is shown a camera drawing of a section of an egg, in which these features are evident. These are very common features during this period of cleavage, and must, it seems to me, be regarded as more or less typical. In two of the cells of Fig. 8 will be observed typical resting nuclei. Whether this is preliminary to the beginning of the later aspects of mitosis I am unable

to say, though it seems altogether probable. At any rate, the facts seem clearly to justify the general conclusion that for a time in the early history of the development of this egg nuclear activity differs very greatly from the ordinary forms of mitosis, and appears to involve direct, or amitotic division. During later cleavage abundant mitosis clearly indicates the prevalence of this form of cell division, if, indeed, it may not wholly supersede the other, though its appearance during regenerative activities shows the possibility of its recurrence under various conditions.

I have called attention to similar nuclear phenomena in the development of *Eudendrium* and *Pennaria*, and in the earlier paper ('04[2], p. 267), cited observations of similar sort from several sources. More recently still other cases have come to light, and it seems altogether probable that as facts multiply and attention is focused upon the phenomena cytologists will be forced to take cognizance of this form of cytogeny and give to it something more than a merely incidental place in cellular activities, and assign to it something more than senescent significance.

Among the more recent data bearing upon this point may be cited, first, the observations of Osborn ('04), in connection with the development of *Fasciolaria ;* and second, similar observations by Glaser ('05), on the same organism, which go to substantially confirm the facts noted by Osborn, though with somewhat different phases of interpretation. Still a third series of facts are brought to light by Child ('04) in a paper on "Amitosis in Moniezia," in which he clearly shows the prevalence of this form of cell division in the growth of the reproductive organs and the development of the sexual cells, and expresses the belief that "future investigation will probably show that amitosis is at least as important in the life of the cell as mitosis."

As I have elsewhere pointed out, it is well known that cell division in Protozoa exhibits very different cytological features than do cleavage cells in early ontogeny.

In many, mitosis seems to be entirely lacking, while in most its features are difficult to correlate with the more typical features in metazoa. Just why we should insist upon finding among a class like cœlenterates all the details of cytogenic mechanics more or less familiar in vertebrates or other higher groups of

metazoa does not appear to be quite obvious. Moreover, why mitosis should have come to be regarded as absolutely cardinal in biologic faith is likewise uncertain. At any rate, the repeated revisions of creed as to centrosome, chromosomes, prelocalization, etc., should suggest a spirit of tolerance toward facts, whatever their significance.

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March 1, 1906.

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Explanation of Plate IX.

Fig. 1. Photograph of section of egg under one-twelfth oil immersion, showing the crescent of pigment granules extending peripherally from the nuclear region. This area is very imperfectly shown as compared with the actual condition as seen under the microscope.

Fig. 2. Photograph of section of an egg about the period of beginning maturation. The flattened nucleus at the outer margin is evident, as is also similar change in the nucleolus. There may also be observed the general dissolution of the nucleus. Magnification as in Fig. 1.

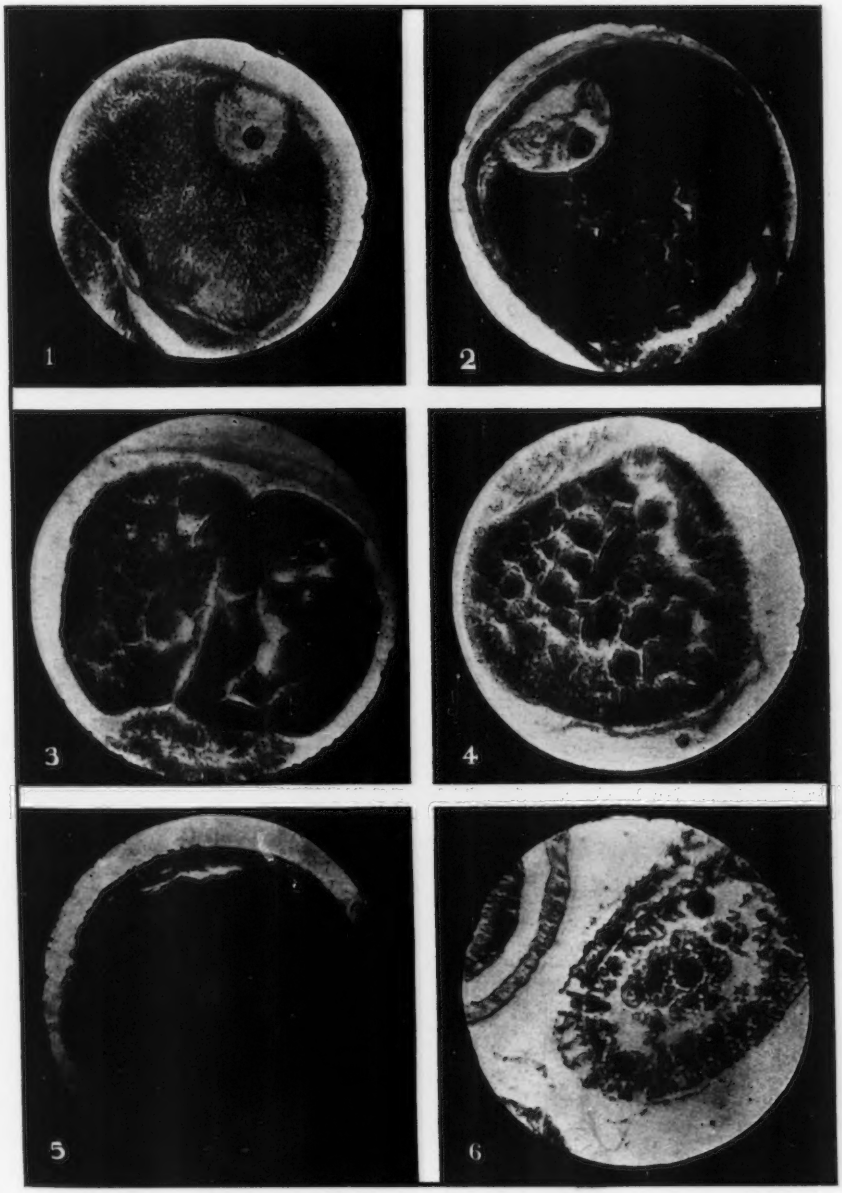
Fig. 3. Photograph of section through two eggs in a single gonophore, showing the flattening of the eggs along the line of contact. The cleavage masses may be observed as about the same stages in each egg. While this is often the case, there are exceptions, as shown in some of the text figures.

Fig. 4. Section of egg photographed under one-twelfth oil immersion, showing the more or less syncytial character of the egg at this stage of development.

Fig. 5. Section through a male gonophore, showing on one side the egg, and on the other the mass of spermatozoa, with the body of the spadix occupying the median region of the section.

Fig. 6. Photograph of portion of an embryo about ready to be liberated, showing the entoderm and ectoderm well differentiated, and with cell-like masses of pigmented yolk spheres in the enteron. It may also be observed that the ectoderm is practically free of pigment matter, as pointed out in the text.

I am under obligation to my colleague, Dr. Rogers, for the photo-micrographs illustrating the above features. All were made under the one-twelfth oil immersion lens, with arc light illumination.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REGARDING THE RATE OF GROWTH OF THE AMERICAN LOBSTER.[1]

PHILIP B. HADLEY.

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At the present time when artificial propagation is bidding fair to at least partially check the ever-increasing depletion of many forms of marine animals whose economic value has long sustained a many-sided fishing industry, any facts which may bear directly or indirectly upon the life, habits or development of such forms might seem to be of value. This is especially true of the American lobster (*Homarus americanus*), a knowledge of whose development must influence not only methods of artificial propagation, which is in these days becoming more common, but also state legislation in determining the size and season at which the taking of lobsters shall be allowable.

The Frequency of Molting and the Percentage of Increase.

The rate of growth of a lobster depends primarily upon two factors, the frequency of the molting periods and the amount of increase in length at each molt. To date, the most complete and only satisfactory account of the development of *Homarus* is presented by Herrick[2] who made many observations at Woods Hole on all of the earlier and many of the later stages. Herrick found that young lobsters (stages 2 to 10) in confinement gain from 11 to 15.84 per cent. at each molt, the average in 66 individuals being 13.5 per cent. He assumes that 15.3 per cent. is the average rate of increase both for the young lobsters which grow up in natural environments, and for adults under normal con-

[1] This paper is presented with the purpose of giving in brief the main facts of a more detailed report on this subject to the Rhode Island Fish Commission. The full account will appear in *The Thirty-Sixth Annual Report of the Rhode Island Commission of Inland Fisheries, 1906.* Reprinted as *Special Paper No. 23, 1906.*

[2] Herrick, *U. S. F. C. Bulletin*, vol. 15, 1895.

ditions. The difference in the above percentages he attributes to the unfavorable conditions of aquarium life. Taking this fact as a basis, and assuming the average length of the first stage lobster to be 7.84 mm., Herrick then constructs the following scheme to show the probable relation between the stage and the size of lobsters from the time of hatching through the thirtieth molt:

Stage.	Length.	Stage.	Length.	Stage.	Length.
1	7.84	11	32.55	21	135.17
2	9.04	12	37.54	22	155.86
3	10.42	13	43.28	23	179.70
4	12.02	14	49.90	24	207.20
5	13.86	15	57.53	25	238.90[1]
6	15.98	16	66.34	26	275.45[2]
7	18.42	17	76.49	27	317.59
8	21.24	18	88.19	28	366.16
9	24.49	19	101.68	29	422.21
10	28.23	20	117.24	30	486.81[3]

[1] 9.5 inches. [2] inches. [3] 19.1 inches.

Regarding the probable *frequency* of molts, Herrick assumes that a lobster molts fourteen to seventeen times during its first year of life, and that in this time it attains a length of two to three inches. From this and other detailed considerations, Herrick finally concludes that a lobster ten and one-half inches long is between four and one-half and five years old, the higher degree of probability being in favor of the lower estimate.

The observations made by the writer and others at the experiment station of the Rhode Island Fish Commission at Wickford, R. I., though differing to some extent from the results obtained by Herrick at Woods Hole, may serve to throw further light on the rate of growth of lobsters in their natural environment, and give some hint as to the conditions which modify it.

The record of the rate of growth of the early stages (one to ten) include observations upon several hundred young lobsters whose definite stage and approximate age was, for the most part, known. Individual records were started immediately after the molt from the third to the fourth stage and were carried on as long as either the weather conditions or the term of life of the young lobsters permitted. In most cases the young individuals were confined in separate compartment cars which furnished a

very natural environment, and rendered it easy to make observations at any time. The facts concerning the development of the early stages may be more tangible when presented in the following table :

WICKFORD LOBSTERS.

Stage.	Length.	Stage-period.	Per cent. Increase.
1	8.2 mm.	2 days	
2	9.6	4 days	17.0
3	11.4	5 days	19.2
4	13.5	12[1] days	18.6
5	16.0	11[1] days	15.0
6	18.8	12.5 days	19.9
7	22 5	14 days	21.0
8	26.5	15.5 days	17.0
9	32.0	21 days	21.0
10	37.9	25 days	17.0
			Total average 18.3

WOODS HOLE LOBSTERS.

Stage.	Length.	Stage-period.	Per cent. Increase.
1	7.84 mm.	1– 5 days	
2	9.20	2– 5 days	17.3
3	11.10	2– 8 days	20.6
4	12.60	10–19 days	13.3
5	14.20	11–18 days	12.7
6	16.10	14 days	13.3
7	18.60		15.5
8	21.03		12.5
9	24.50		16.5
10	28.03		14.0
			Total average 15.3[2]

[1] The fifth stage-period is often shorter than the fourth because the water at Wickford is usually the warmest during the fifth stage-period.

[2] This percentage is greater than in the case of another group of 66 lobsters in which Herrick obtained a result of 13.67 per cent.

The foregoing demonstrates the fact that the average stage-period of the Wickford lobsters is less than the stage-period of the Woods Hole lobsters. Further that, while the percentage of increase at each molt for the Woods Hole lobsters (kept in aquaria) was only 13.5 or 15.3, the amount of increase for the Wickford lobsters was over 18 per cent. in the individuals recorded above, while in the case of a group of individuals which had been especially selected (*i. e.*, the smaller and weaker specimens were

thrown out) the average amount of increase for the first ten stages was 20.9 per cent. In this last instance the lobsters in the respective stages were consequently much larger than those in the group of Wickford lobsters tabulated above. Their average measurements, however, are presented in the following table:

Stage.	4	5	6	7	8	9	10
Length (mm.).	14.4	17.0	20.5	24.6	31.3	37.0	45.0
Stg.-per. (days).	11.7	11.2	12.2	13.5	15.1	21.0	25.1
Per cent. increase.		18.0	20.6	20.0	27.2	18.5	21.6

These and other observations would demonstrate that there are great variations in the rate of development of lobsters, not only in different localities, and under different conditions of environment, but also in the same locality and under identical conditions. Furthermore, that there is a tendency manifested in those individuals which are slightly above the normal in size and strength, to increase the advantage which they have already gained. This advantage, lodged in the fourth stage lobster, may be no more than a millimeter, but this slight gain compounded through numerous successive stages gives, even the tenth and eleventh stage, a decided lead which is never again lost and which may be observed in the last mentioned group.

Continued observations upon the later stages (from the tenth on) prove that not twelve to seventeen stages, as calculated by Herrick, but an average of twelve stages are passed during the first year of the lobster's existence. We may trace the future development of the young lobster through the later successive stages as follows: September finds the average individual, hatched the previous June, in the ninth stage and with an average length of 32 mm. He passes into the tenth stage in the latter part of September, with a corresponding length of 37.9 mm. In the latter part of October or the first of November he enters the eleventh stage with an increase to 45 mm. Through the months of November, December, January, February and March he lies dormant, passing into the twelfth stage some time in April or the first part of May. Thus it appears that a lobster one year old is in the twelfth stage and has an average length of 53 mm. There are always exceptions to this rule, — instances where an individual

may occasionally pass into the twelfth stage before the winter months. Such specimens sometimes manifest an increase of 28 per cent. in passing a single stage. These lobsters are, however, usually among those which were hatched early in the season, and are not very common.

From observations upon the yearling lobsters it becomes apparent that the young creature molts on the average of four times during its second year of life. The thirteenth stage is entered some time in July or August, with a corresponding length of 62 mm. In the latter part of August he molts for the thirteenth time and now covers 73 mm. The entrance to the fifteenth stage occurs in October of the second year. No further change takes place until the following April; that is to say that the average lobster passes its second winter in the fifteenth stage, length 86 mm. (3⅜ inches). By the middle of June we find the young lobster, now approximately two years old, in the sixteenth stage, and with a length of 102 mm. ($4\frac{1}{12}$ inches).

Observations on the molting periods of lobsters over two years old make it apparent that the entrance to the seventeenth stage takes place some time in the late summer of the third year. The lobster generally molts again before the winter months of the same year into the eighteenth stage with a length of 141 mm. (5⅝ inches). No further change is experienced until the following April.

After the seventeenth or eighteenth stage the percentage of increase at each successive molt undergoes a gradual diminution as the molting periods become less frequent. The amount of increase for lobsters about 6 inches in length appears to be in the neighborhood of 15 per cent. Thus continuing, we find that the young lobster passes its third winter in the eighteenth stage, molts again in the spring (usually in April) and by June, when approximately three years old, has a length of 162 mm. (6½ inches).

In lobsters of 7 inches and over we find a still smaller percentage of increase at each molt; 11 or 12 per cent. represents with a fair degree of accuracy the average percentage of increase in length for lobsters between 7 and 10 inches.

Further observation reveals the fact that lobsters over 6 inches

in length do not molt oftener than twice in a year; once in the spring or early summer and once in the autumn. Thus the average lobster enters the twentieth stage some time in the autumn of his fourth year and at this molt increases from 162 mm. to 181 mm. (7¼ inches). In the late spring or early summer of the following year the lobster, now approximately four years old, enters the twenty-first stage with a corresponding length of 200 mm. (8 inches).

If the case is not one of a young female bearing external eggs (very rare in lobsters of this length), we may expect another molt the following autumn and consequently find the lobster in the twenty-second stage now with a length of 222 mm. (8⅞ inches). In all probability the molting periods of the male and female remain the same until past the nine-inch length. Therefore, the entrance to the twenty-third stage probably takes place just before, or at any rate soon after, the lobster becomes five years old. The corresponding length is 247 mm. (9⅞ inches).

By the time this length is reached many of the female lobsters are sexually mature and are bearing external eggs. Owing to this fact, from this time on the rate of growth of the females must be much diminished. This is due no doubt to the checking of the growing process, a phenomenon which very naturally precedes the spawning period; also to the length of time (ten to eleven months) the eggs are carried. The male lobsters, on the other hand, maintain their former rate of development so that by the twenty-fourth stage the average male lobster has a length of 275 mm. (11 inches) and cannot be much less than six years old. In the case of the females, however, which have borne eggs since the nine-inch stage, the eleven-inch limit cannot be attained in a shorter period than eight years.

This discrepancy in the rate of growth of the male and female lobsters from this time on, is undoubtedly the explanation of the fact that, in nearly all individuals in which the sex has been observed, the "giant" lobsters have been of the male variety. There are few data on the rate of growth of large lobsters but it is probable that after the ten-inch size has been attained, the lobster does not molt oftener than once in a year; and after the fifteen-inch stage not oftener than once in two years. Regarding

Stage No.	Sex.	Age.	Length, mm.
1	Male and		8.2
2	Female.	3 days.[1]	9.6
3	"	7 days.	11.4
4	"	12 days.	13.5
5	"	24 days.	16.0
6	"	36 days.	18.8
7	"	7 weeks.	22.5
8	"	9 weeks.	26.5
9	"	3 months.	32.0
10	"	5 months.	37.9
11	"	9 months.	45.0
12	"	1 year.	53.0
13	"	1 yr. 1 mo.	62.0
14	"	1 yr. 3 mo.	73.0
15	"	1 yr. 6 mo.	86.0
16	"	2 yrs.	102.0
17	"	2 yrs. 3 mo.	121.0
18	"	2 yrs. 6 mo.	141.0
19	"	3 yrs.	162.0
20	"	3 yrs. 6 mo.	180.0
21	"	4 yrs.	200.0
22	Male.	4 yrs. 6 mo.	222.0
"	Female.	4 yrs. 6 mo.	"
23	Male.	5 yrs.	247.0
"	Female.	6 yrs. 5 mo.[2]	"
24	Male.	6 yrs.	275.0[3]
"	Female.	8 yrs. 4 mo.	"
25	Male.	7 yrs.	300.0
"	Female.	10 yrs. 4 mo.	"
26	Male.	8 yrs.	327.0
"	Female.	12 yrs. 4 mo.	"
27	Male.	9 yrs.	356.0
"	Female.	14 yrs. 4 mo.	"
28	Male.	10 yrs.	380.0
"	Female.	16 yrs. 4 mo.	"
29	Male.	12 yrs.	406.0
"	Female.	18 yrs. 4 mo.	"
30	Male.	14 yrs.	431.0
"	Female.	20 yrs. 4 mo.	"
31	Male.	17 yrs.	457.0
32	"	20 yrs.	480.0
33	"	23 yrs.	505.0
34	"	26 yrs.	525.0
35	"	29 yrs.	546.0
36	"	33 yrs.	568.0[4]

[1] Age at entrance to the stage.

[2] Assumes that the lobster spawns for the first time in the summer of its sixth year, and that the eggs hatch the following summer.

[3] 11 inches. [4] 22¾ inches.

the growth of "giant" lobsters, it appears reasonable to believe that the molting process does not occur oftener than once in three years; and this is a small estimate. The amount of increase in these specimens at a single molt cannot be over four or

five per cent. and is often inappreciable. The shells of these huge lobsters present every appearance of great age and give testimony to a life of inactivity. Using as a basis the observations which led to the foregoing conclusions, the writer has compiled a table showing the estimated rate of development of lobsters from the time of hatching to the attainment of the greatest known size. While the data on the first twenty stages have their ground in actual observation, the records of the later stages have been deduced from less positive evidence, and are, to a great extent, speculative. The great variation in the size of lobsters, even of the same age and stage, render it well-nigh impossible to tell off-hand the age of any adult lobster. On the other hand, the size of large numbers of individual lobsters of a certain age must remain not far from a general average, on a basis of which, the approximate age of large numbers of individuals can be determined with a fair degree of certainty. It is this average, together with the correlated age, that the writer has attempted to formulate in the preceding table:

Influences on the Rate of Growth.

Among the influences which modify the rate of growth of young lobsters under natural or artificial conditions, are to be mentioned especially the following: temperature, food supply, light, parasites, injuries and individual physiological peculiarities. It is probable that the water temperature and physiological condition are the most influential for young lobsters in the ocean. The others enter largely into consideration in the problems of artificial propagation. The frequency of the molting periods and, secondarily, the amount of increase in length at each molt, is directly dependent upon and determined by the prevailing temperature of the water; a difference of twelve degrees may cause the period of growth to the fourth stage to be over twice as long as normally. Thus we find great variations in the rates of growth of lobsters at different points on the Atlantic coast. For this reason it is very probable that the lobsters in the warm waters of Narrangansett Bay may attain marketable size (10½ inches in Massachusetts) much sooner than do the Massachusetts or Maine lobsters.

It is apparent through other observations that the effects of strong lights and, as shown by Emmel,[1] the mutilation of appendages, exert an influence detrimental to the development of the lobster in the early stages. Excessive sunlight in cases where the lobsters were exposed superficially in the water, appears to cause not only a marked increase in the duration of the stage-periods, but also a diminution in the percentage of increase in length at molts; and furthermore, a generally less healthy condition in the lobsters themselves. This may be brought about either directly, by inhibiting the body processes and general metabolism, or indirectly, by favoring the excessive growth of diatoms, algæ and protozoa which, under certain conditions, may accumulate on the body and appendages, to such an extent as to prevent nearly all activity. It is also observable in this connection, that food supply may play an important rôle in determining the size of the young lobster.

[1] V. E. EMMEL; "The Regeneration of Lost Parts in the Lobster," *The Thirty-Fifth Annual Report of the Rhode Island Commission of Inland Fisheries, 1905.*

NOTE ON THE INFLUENCE OF TEMPERATURE UPON THE RATE OF THE HEART-BEAT IN A CRUSTACEAN (CERIODAPHNIA).[1]

T. BRAILSFORD ROBERTSON.

1. Arrhenius has shown[2] that the velocity of a chemical reaction increases very much more rapidly with increasing temperature than any known physical phenomenon. The velocity of a chemical reaction increases about 10 per cent. per degree centigrade rise in temperature while molecular velocity, the electric conductivity of a wire, the elasticity of a solid, the viscosity of a fluid, surface-tension, etc., are much less affected by the same rise of temperature. The viscosity of a fluid, for example, only diminishes about 2 per cent. per degree centigrade rise in temperature. The viscosity of a gas, according to Maxwell's equation for air[3] should increase about 6.7 per cent. per degree centigrade rise in temperature at 15° C.

2. The fact of the extreme variability of chemical reactions with temperature has been applied to ascertain whether given biological phenomena involve chemical reactions or not. Arrhenius[4] and van t'Hoff[5] have shown that the quotient

$$\frac{\text{velocity of reaction at } T_{n+10}}{\text{velocity of reaction at } T_n}$$

is equal to about 2, T_n being the absolute temperature. That is to say, the velocity of a chemical reaction increases about 100 per cent. per ten degrees. Hertwig[6] has found that the rate of development of frog's eggs increases very rapidly with rise of temperature. At Dr. Loeb's suggestion C. D. Snyder[7] investi-

[1] From the Rudolph Spreckels Physiological Laboratory of the University of California.

[2] *Zeitschrift f. Physik-Chemie*, 1899, Bd. 4, s. 226.

[3] "Theory of Heat," 1872, p. 279.

[4] *Loc. cit.*

[5] "Études de dynamique chimique," p. 112, etc.

[6] "Die Zelle und die Gewebe," Bd. II., S. 119.

[7] University of California Publications, Physiology, Vol. 2, p. 125.

gated the influence of temperature upon the rate of the heart-beat and found that the rate of the beat in the heart of the terrapin (*Clemmys marmorata*) is almost exactly doubled by ten degrees rise in temperature between the temperatures 10° C. and 32.5° C. — at lower temperatures the rate is more than doubled by a rise of 10° while at higher temperatures the rate is somewhat less than doubled by the same rise in temperature. Loeb[8] has found that the velocity of artificial maturation in the eggs of Lottia is more than doubled by raising the temperature from 8° C. to 18° C. Other investigations on the influence of temperature upon biological phenomena are being carried out in this laboratory. These observations do not prove that the above-mentioned biological phenomena are entirely chemical in character, but they afford indication that chemical reactions are involved although not to the exclusion of possible concurrent physical changes.

3. It appeared to me of interest, in connection with experiments on the influence of electrolytes on the rate of the heart-beat, of which an account will appear at an early date, to ascertain the influence of temperature upon the rate of the heart-beat in the transparent fresh-water crustacean Ceriodaphnia (?). The organism, after washing in tap-water, was laid in a drop of tap-water in the depression on the glass top of an Englemann gas-chamber, the temperature being regulated by running warm or cold water through the chamber. A thermometer was fitted into the chamber so that the bulb lay directly under the depression in which the organism was placed. A few minutes was always allowed to elapse before the rate of the beat was registered in order to allow the organism to attain the same temperature as the bulb of the thermometer. The beats at room-temperature and at higher temperatures are so rapid that they cannot be counted but have to be recorded by tapping a key which completes a circuit including a signal-magnet, which thus registers a mark upon a revolving drum for every beat, the time being taken with a stop-watch.

4. The following are the experimental results.

[8] University of California Publications, Physiology, Vol. 3, p. 1.

EXPERIMENT 1.

Temperature.	Beats per Second.
24.75°	6.53
20.75°	4.43
15.75°	2.47

EXPERIMENT 2.

Temperature.	Beats per Second
15°	2.53
19°	3.46
23°	4.39
27°	6.11

EXPERIMENT 3.

Temperature.	Beats per Second.
15°	2.63
17°	2.95
19°	3.67
21°	4.87
23°	5.05
25°	6.59
27°	6.10
29°	7.375

$$\frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 2.51; \quad \frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 2.07; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.01.$$

EXPERIMENT 4.

Temperature.	Beats per Second.
13°	2.22
15°	2.88
17°	3.46
19°	2.95
21°	3.91
23°	5
25°	5.71
27°	6.23
29°	6.15

$$\frac{\text{Rate at } 23^\circ}{\text{Rate at } 13^\circ} = 2.25; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 1.98;$$

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 1.80; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.08.$$

EXPERIMENT 5.

Temperature.	Beats per Second.	Remarks
11°	2.31	Irregular pauses in diastole accompanied by very slight quick beats.
13°	——	Still irregular.
15°	4.12	Regular.
19°	4.79	"
21°	6.15	"
25°	6.22	"
29°	6.17	"
31°	7.01	Gills stopped

At 33° the whole animal went into convulsive tremors, and at 35° the heart stopped permanently.

$$\frac{\text{Rate at } 21°}{\text{Rate at } 11°} = 2.66\,; \quad \frac{\text{Rate at } 25°}{\text{Rate at } 15°} = 1.51\,;$$

$$\frac{\text{Rate at } 29°}{\text{Rate at } 19°} = 1.29\,; \quad \frac{\text{Rate at } 31°}{\text{Rate at } 21°} = 1.14.$$

EXPERIMENT 6.

Temperature.	Beats per Second.	Remarks.
11°	2.11	Beat irregular
13°	2.53	" "
15°	2.53	" regular
19°	2.67	" "
21°	4.05	" "
23°	4.80	" "
25°	5.56	" "
29°	6.30	" "
29°	5.33	After a fall and subsequent rise in temperature.

$$\frac{\text{Rate at } 21°}{\text{Rate at } 11°} = 1.92\,; \quad \frac{\text{Rate at } 23°}{\text{Rate at } 13°} = 1.90\,; \quad \frac{\text{Rate at } 25°}{\text{Rate at } 15°} = 2.20\,;$$

$$\frac{\text{Rate at } 29°}{\text{Rate at } 19°} = 2.36\,; \quad \frac{\text{Rate at } 29°}{\text{Rate at } 19°} \text{ (second observation)} = 2.00.$$

EXPERIMENT 7.

Temperature.	Beats per Second.
11°	1.64
15°	2.38
17°	2.63
19°	2.98
*21°	4.53
21°	4.55
21°	4.48
25°	5.33
27°	5.4
29°	5.15

$$\frac{\text{Average rate at } 21°}{\text{Rate at } 11°} = 2.76\,; \quad \frac{\text{Rate at } 25°}{\text{Rate at } 15°} = 2.24\,;$$

$$\frac{\text{Rate at } 27°}{\text{Rate at } 17°} = 2.05\,; \quad \frac{\text{Rate at } 29°}{\text{Rate at } 19°} = 2.06.$$

The beat was regular throughout the observations in this experiment. The observations were made in the following order,

21°, 21°, 29°, 27°, 25°, *21°, 19°, 17°, 15°, 11°—hence the rate of beat at 21° was counted three times, twice at the beginning of the experiment and once after taking the animal through a series of temperature-changes none of which were extreme — it will be observed that the three observations at 21° are in very good agreement.

EXPERIMENT 8.

Temperature.	Beats per Second.
11°	1.82
15°	2.95
17°	3.78
19°	4.09
*21°	4.375
21°	4.15 } Average = 4.40
21°	4.65 }
25°	4.67
27°	6.67

$$\frac{\text{Average rate at } 21°}{\text{Rate at } 11°} = 2.41\,; \quad \frac{\text{Rate at } 25°}{\text{Rate at } 15°} = 1.58\,;$$

$$\frac{\text{Rate at } 27°}{\text{Rate at } 17°} = 1.76.$$

The beat was regular throughout the experiment. The organism was taken through the following temperatures in the following order: 21°, 21°, 29° (beats not counted), 27°, 25°, *21°, 19°, 17°, 15°, 11°—hence the rate of beat at 21° was counted three times, twice at the beginning of the experiment and once after taking the animal through a cycle of temperature-changes none of which were extreme—the three determinations of the rate at 21° are in good agreement with each other and with those in experiment 7.

EXPERIMENT 9.

Temperature.	Beats per Second.
19°	3.27
21°	5.12
29°	6.25

The organism was taken through the following temperatures in the following order, 31° (not counted), 29°, 21° and 19°.

$$\frac{\text{Rate at } 29°}{\text{Rate at } 19°} = 1.91.$$

EXPERIMENT 10.

Temperature.	Beats per Second.
29°	6.15
21°	3.06
21°	3.23
19°	2.86

$$\frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.15.$$

The beat was regular throughout, but the temperature had been raised above 29° before the observations at 21° were taken.

EXPERIMENT 11.

Temperature.	Beats per Second.
17°	2.38
21°	4.04
27°	5

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 2.10.$$

The observations were taken in the following order, 21°, 17°, 27°.

EXPERIMENT 12.

Temperature.	Beats per Second.
13°	2.28
21°	4.57
23°	5

$$\frac{\text{Rate at } 23^\circ}{\text{Rate at } 13^\circ} = 2.19.$$

5. The average value of the coefficient $\frac{\text{Rate at } T_{n+10}}{\text{Rate at } T_n}$ for all the observations made was 2.03. The results are therefore such as to confirm Snyder's observations, referred to above, in so far as they apply in general to rhythmically contracting tissues and to lead us to conclude that a chemical reaction is involved — as I have assumed in previous papers.[1]

6. The rate of the beat in different individuals at 21° is remarkably constant, provided they have been treated in the same manner and have not been subjected to any extreme temperatures — this is particularly well shown in experiments 7 and

[1] *Transactions of the Royal Society of South Australia*, Vol. XXIX., p. 47; *Plüger's Arch. f. d. ges. Physiologie*, Bd. 110, s. 610.

8. I have repeatedly confirmed the fact that the rate of the beat at 21° is about 4.5, with a possible variation of about 5 per cent. The organisms were all taken from the same vessel, in which they were kept; but they differed widely in size and development, some containing eggs and others containing well-developed embryos. Ceriodaphnia therefore affords homogeneous material for experiments on the heart-beat.

TWO DISTOMES.

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1. PNEUMONŒCES COMPLEXUS, N. S., a NEW FROG DISTOME.

This description is based on the examination of several preserved specimens which were received from North Carolina marked "From the mouth of *Rana pipiens*."

P. complexus is a rather elongated worm, between 5 mm. and 8 mm. in length, and in the widest portion, about 1.7 mm. wide. The thickness is about .71 mm., a cross-section being elliptical in outline. The widest region is just forward of the middle, and from here it tapers very slightly toward the hinder end, which is rounded and blunt. Toward the forward end it tapers more rapidly, sometimes giving the worm almost the appearance of having a neck. The oral sucker is subterminal and .4 mm. in diameter. The acetabulum is 2.4 mm. from the anterior end of the animal and very slightly smaller than the oral sucker, being .38 mm. in diameter. Both suckers are sessile. All the specimens examined were smooth, *i. e.*, without spines. This may have been due, however, to maceration. Of the five species of this genus described by Stafford (1902), all are covered with spines except one, *P. longiplexus*, which is smooth. The determination of this point with respect to *P. complexus* will depend finally upon the examination of a living or freshly-killed specimen. In the specimens examined, however, there were patches where the cuticle seemed to be intact. It was very thick and there was no trace of spines. Thus the evidence seems almost conclusive that the worm is a smooth one.

The mouth is easily discernible in the center of the oral sucker, and leads to the muscular, bulb-like pharynx. The œsophagus is about .5 mm. in length. It then branches, forming the two intestinal cæca, which extend to within .4 mm. of the posterior end of the body. The cæca are rounded tubes about .15 mm. in diameter, and are without branches.

The excretory vesicle, which is tubular and in cross-section about the size of one of the intestinal cæca, is extremely long. The median portion extends from the excretory pore, which is terminally located at the posterior extremity, to a position just posterior to the acetabulum. Here it branches, forming the two crura, which extend outward and forward to about the plane of the posterior end of the œsophagus. The excretory vesicle is extremely thin-walled, and almost impossible to be seen except in sections. It is dorsal in position throughout its entire extent.

The testes are two large, very prominent organs just posterior to the middle of the animal. They are irregularly ovate in outline and are somewhat lobed. They do not both lie in the same plane, the left one usually being posterior to, but overlapping, the right. The posterior one is usually somewhat longer than the anterior, being 1.4 mm. long, while the anterior one is 1.1 mm. long. Each is about .92 mm. wide and about .78 mm. thick. The vas deferens from the left testis runs forward to the left of the right testis, lying between it and ovary. Just anterior to the shell gland it is joined by its fellow from the right testis, and from here a slightly convoluted tube extends to the genital pore. There is no cirrus or cirrus sac. The genital pore is a minute opening on the ventral side, in the same transverse plane as the pharynx and to the right of the median plane.

The ovary is a somewhat elongated organ considerably smaller than either testis. It is usually on the left side and slightly posterior to the acetabulum. Occasionally it is on the right side, and when it is so placed the testis on that side is posterior to the other. It is about .7 mm. in length, .32 mm. in width, and .32 mm. thick (dorso-ventrally). The oviduct runs from the ovary into the shell-gland where there is an ootype. The shell-gland lies in the middle of the body just posterior to the acetabulum.

The receptaculum seminis is a sac about one-half as large as the ovary. It lies just forward of and lateral to the right testis. Its duct runs forward and to the left, meeting the oviduct at its entrance into shell gland. There is no Laurer's canal.

The yolk glands are numerous, being placed in clusters along both sides of the body from the position of the pharynx to the posterior extremity of the hinder testis. There are five or six

distinct groups or clusters on each side, each group being composed of 6 to 20 glandular bodies. They are ventrally placed and follow the general course of the intestine.

The tube from the forward clusters on each side meets that from the rear clusters just inside the intestinal cæcum and in a transverse plane just posterior to the ootype. The transverse tubes meet near the median plane, well toward the dorsal body-wall, and from here a short tube leads to the yolk reservoir.

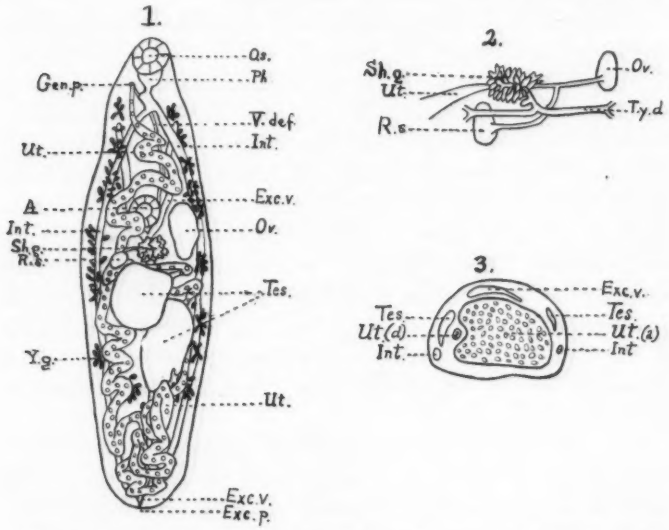

FIG. 1. *Pneumonœces complexus*, ventral aspect. *O. s.*, oral sucker; *A.*, acetabulum; *Ph.*, pharynx; *Int.*, intestinal cæcum; *Exc. v.*, excretory vesicle; *Ut.*, uterus; *Tes.*, testes; *R. s.*, receptaculum seminis; *Gen. p.*, genital pore; *Ov.*, ovary; *Sh. g.*, shell gland; *V. def.*, vas deferens; *Y. g.*, yolk gland.

FIG. 2. Diagram of female genital organs in *P. complexus*. *Ov.*, ovary; *Sh. g.*, shell-gland; *Ut.*, uterus; *R. s.*, receptaculum seminis; *T. y. d.*, tranverse yolk duct.

FIG. 3. Cross-section of *Renifer elongatus* in region of testes. *Ut.* (*d*), uterus, descending; *Ut.* (*a*), uterus, ascending; *Tes.*, testis; *Int.*, intestinal cæcum; *Exc. v.*, excretory vesicle.

From the yolk reservoir a tube extends diagonally forward and towards the ventral body wall joining the oviduct after its entrance into the shell gland. The yolk reservoir is a pear-shaped sac about .12 mm. in length.

The uterus is a continuation of the oviduct, running to the posterior end of the animal, then forward to the genital pore. It winds about, filling the spaces between the other organs, and in the case of adult worms often nearly covers them. It is circular in cross-section, and about .15 mm. in diameter. In the adult animal this tube is filled with eggs which as they ripen take on a dark brown color, and give the worm a peculiar mottled appearance. There are no lateral longitudinal folds as in the case of most of the other species of this genus.

P. complexus is closely related to *P. similiplexus*, described by Stafford (1902) under the name of *Hæmatolœchus similiplexus*. The principal differences between the two species are that in *P. complexus* the testes are very much larger than in *P. similiplexus;* there are no lateral longitudinal folds of the uterus; and the eggs are considerably smaller, being .029 mm. by .014 mm. as compared with .039 mm. by .019 mm. in *P. similiplexus*. In outer characters, — size, shape, suckers, etc., — there is a close similarity between the two species. *P. similiplexus* is covered with spines, while *P. complexus* is probably smooth. In this respect *P. complexus* resembles *P. longiplexus*. In respect to the folds of the uterus it most closely resembles *P. medioplexus*. The ascending and descending coils seem however to have less regularity that in that species.

The other species of this genus are universally reported as being found in the lungs of frogs, while, as has already been noted, these specimens were labelled as having been found in the mouth of the leopard frog. Upon inquiry I found that these distomes are rarely or never found in the mouth of a living or freshly-killed animal. If, however, the frogs are killed by chloroform, and especially if they are left for some time in the killing jar, the worms, still alive, may frequently be found in the mouths of the dead animals. They may be thrown from the lungs by the struggles of the dying animal, or they may find their way to the mouth in their efforts to escape from a dead host. At any rate the above facts explain their being found in the mouth of the frog, and leave every probability that *P. complexus* is a lung parasite.

II. Renifer elongatus Pratt.

This worm was first described by Pratt (1903) from a single specimen, mature but not yet fully grown. The object here is simply to supplement that description, by a comparison with a full-grown specimen. The worm from which the following measurements were taken was selected as the largest of about 40 or 50 specimens at hand. This worm is 5.5 mm. in length, and 1.7 mm. wide at the widest part, while that described by Pratt was 3 mm. in length, and .68 mm. in width. The thickness of the two worms is .93 mm. and .4 mm., respectively.

The oral sucker is round and about .35 mm. in diameter; the acetabulum is decidedly elliptical in outline, extending .82 mm. transversely and .6 mm. longitudinally. The corresponding measurements given by Pratt are the following: oral sucker; length, .32 mm., width, .33 mm.; acetabulum; length, .4 mm., width, .36 mm. The relative positions of the suckers on the animal are of course the same. The relative sizes of the various parts of the digestive tracts of the two worms correspond to relative sizes of the worms themselves.

The most marked difference between the two worms are the greatly increased size of the ascending limb of the uterus, and the consequent displacement of the other organs in the larger worm. In the region just back of the testes, the uterus occupies nearly two thirds of the space within the body walls, and the excretory vesicle is pressed flat against the dorsal wall, extending from one lateral margin to the other. In the region of the testes there is but little diminution in the size of the uterus, and the testes are pressed outward toward the lateral body walls, they themselves being apparently somewhat flattened (see Fig. 3). Forward of the acetabulum, the uterus rapidly decreases in size as it approaches the genital pore.

The eggs were approximately the same size as those in the smaller worm. The measurements given by Pratt are .035 mm. by .02 mm. The measurements in the larger worm were .034 mm. by .021 mm.

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